SUPPLEMENTAL DATA

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Patient selection, skin biopsies and cell culture
Patients were recruited from the Norwegian MODY Registry (1-5) and Joslin Diabetes Center. Some fibroblasts (AG16102 and GM1237) were purchased from Coriell Institute for Medical Research (Camden, NJ, USA). GM1237 is a MODY1 patient separate from the MODY1 family whom we have independently recruited from Norway. We selected numerous patients with disease-causing variants affecting a single nucleotide base to facilitate future *in vitro* correction of the genetic variant. We devised a family node design of two to four closely related subjects (a duo, trio or quadro design) to obtain appropriate controls. This takes advantage of the shared environment of a family node and also the similar genetic background of siblings and their parents. This study design also provides the opportunity to study subjects with disease-causing variants both prior to and after the development of clinically manifest diabetes.

A 4 to 6 mm skin biopsy was taken from the anterior aspect of the upper forearm, trimmed, cut into pieces and cultivated in Amniochrome™ II Complete Medium (with supplement) (Lonza, Verviers, Belgium) at 37 °C with 5 % CO_2. Fibroblasts growing out from the explant were maintained in Amniochrome™ II (without supplement) (Lonza, Verviers, Belgium). Skin fibroblasts were subsequently cultured in DMEM with L-glutamine, 1 g/L glucose (Mediatech Inc, Manassas, VA, USA), 10 % FBS, MEM sodium pyruvate (Life Technologies™, Grand Island, NY, USA) and penicillin-streptomycin (Mediatech Inc, Manassas, VA, USA) (6). hiPSCs were cultured in DMEM/F-12 with 15 mM HEPES (STEMCELL Technologies Inc, Vancouver, BC, Canada), 20 % KnockOut™ serum replacement (KOSR), L-glutamine, MEM non-essential amino acids (Life Technologies™, Grand Island, NY, USA), penicillin-streptomycin (Mediatech Inc, Manassas, VA, USA), and supplemented with 10 ng/ml basic growth factor (bFGF) (Miltenyi Biotec, Cambridge, MA, USA). hiPSCs were either grown on irradiated CF-1 mouse embryonic fibroblasts (MEFs) or in mTeSR®1 feeder-free media (STEMCELL Technologies Inc, Vancouver, BC, Canada). hiPSC media was replaced every 24 h.

Sequencing of MODY genes
hHnf4aseqF: 5' – GCACCAGCTATCTTGCCAAC – 3';
hHnf4aseqR: 5' – AGGAGAAGTCTGGCAGAGCG – 3'
hGckexon5seqF: 5’ – GTAGAGCAGATCCTGGCAGAG – 3’;
hGckexon5seqR: 5’ – CTGAGCCTTCTGGGGTGGAG – 3’;
hHnf1aexon4seqF: 5’ – GGCGGAATGCATCCAGAGAG – 3’;
hHnf1aexon4seqR: 5’ – CGTGGACCTTACTGGGGGAG – 3’;
hHnf1bexon2seqF: 5’ – TGAGGACCCTTGGAGGGCTG – 3’;
hHnf1bexon2seqR: 5’ – GTCGGAGGATCTCTCGTTGC – 3’;
hCELexon11F: 5´ – TCCCTCACTCATTCTTCTATGGCAAC – 3´;
hCELexon11R: 5´ – TCCTGCAGCTTAGCCTTGGG – 3´;
hCELexon11seqF: 5’ – CACACACTGGGAACCCT – 3´;
hCELexon11seqR: 5’ – TCCTGCAGCTTAGCCTTGGG – 3´.

Production of VSV-G pseudotyped lentivirus
Human STEMCCA Cre-excisable constitutive polycistronic (OKSM) lentiviruses were prepared by five plasmid co-transfection of 293FT cells. EF1α – hSTEMCCA-LoxP (OKSM) lentiviral vector (a polycistronic lentiviral vector expressing a human “stem cell cassette” with the four reprogramming factors *OCT4*, *KLF4*, *SOX2* and *CMYC*), tat, rev, gag/pol and vsv-g plasmids were kindly provided by Dr. Gustavo Mostoslavsky (7). Briefly, 6 x 15 cm plates of 293FT cells were transfected with the five plasmids (ratio of 20:1:1:1:2) using calcium phosphate transfection method (Clontech Laboratories Inc,

Mountain View, CA, USA). Culture media was discarded 24 h post-transfection and replaced with fresh media. Twenty four h later, the supernatant was harvested and this process was repeated after a further 24 h. The lentiviral supernatant was centrifuged at 1000 rpm for 5 min to remove cell debris and then filtered with a 0.45 μm filter (EMD Millipore, Billerica, MA, USA) to remove residual debris. Lentiviruses were pelleted by centrifuging at 23000 rpm for 90 min at 4 °C. The lentiviral pellet was resuspended in 1.2 ml of fibroblast media and frozen at -80 °C in aliquots. Lentiviruses were subsequently titred using Lenti-X™ p24 rapid titre kit (Clontech Laboratories Inc, Mountain View, CA, USA).

Generation of hiPSCs from patient skin fibroblasts

Skin fibroblasts (3×10^4) were plated in each well of a 6 well plate, and virally transduced with the lentiviral particles in the presence of 5 μg/ml Polybrene® (EMD Millipore, Billerica, MA, USA) after 8 h. The fibroblasts were washed three times with PBS and fed with fresh fibroblast media after 24 h. On day 5, fibroblasts were trypsinized (0.05 % trypsin) and approximately 3×10^4 fibroblasts were seeded into each well of a 6 well plate containing irradiated CF-1 mouse embryonic fibroblasts (MEFs). From day 7 onwards, the culture media was replaced daily with hiPSC media supplemented with 10 ng/ml of basic fibroblast growth factor (bFGF). hiPSCs emerged following two to three weeks in culture, and were individually picked, cultured, expanded and frozen. They were subsequently characterized.

Quantitative real time PCR (qRT-PCR)

Total RNA was extracted using the RNeasy Mini Kit (QIAGEN Inc, Valencia, CA, USA). Two μg of total RNA was reverse transcribed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Bedford, MA, USA). Twenty five ng of cDNA equivalent was used in a qPCR reaction. Primers used are as follows:
hOct43utrF: 5’ – ACACAAAGGGTGGGGGCAGGGGA – 3’;
hOct43utrR: 5’ – ACCTTCCCTCCAACCAGTTGCCCCA – 3’;
hNanogF: 5’ – GACCTGGTGCACCCAATCCT – 3’;
hNanogR: 5’ – TCCAAGGCAGCCTCCAAGTC – 3’.
For copy number viral integration determination, 10 ng of gDNA was used in a qPCR reaction. Primers used are as follows:
hgenomicFoxA2F: 5’ – GCGACCCCAAGACCTACAG – 3’;
hgenomicFoxA2R: 5’ – GGTTCTGCCGGTAGAAGGG – 3’;
hgenomicSox2F: 5’ –CAACTCGGAGATCAGCAAGC – 3’;
hgenomicSox2R: 5’ –GGCAGCGTGTACTTATCCTTC – 3’.

Immunostaining

hiPSCs grown in 12 well plates were fixed with 4 % paraformaldehyde for 20 min at room temperature. They were washed three times with PBS or PBST (0.1 % Triton-X) and blocked with 5 % donkey serum for 1 h on ice. Cells were then incubated with primary antibody [OCT4 (1:100; sc-5279; Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA), SOX2 (1:200; ab97959; Abcam, Cambridge, MA, USA), NANOG (1:100; AF1997; R&D Systems Inc, Minneapolis, MN, USA), SSEA-4 (1:100; 01554) and TRA-1-60 (1:100; 01555; STEMCELL Technologies Inc, Vancouver, BC, Canada)] overnight at 4 °C. They were then washed three times with PBS or PBST and incubated with the appropriate secondary antibodies [(1:200; 711-516-150; 711-516-152; 715-546-150; Jackson ImmunoResearch Laboratories Inc, West Grove, PA, USA) and (1:200; A11058; Life Technologies™, Grand Island, NY, USA)] for 1 h at room temperature. The cells were then incubated with DAPI (1:5000) in PBST for 15 min, washed twice with PBST and then imaged. In instances whereby hiPSCs were co-stained, they were sequentially stained with cell surface antibodies followed by nuclear staining and then DAPI.

Teratoma assay

Approximately 1 to 2×10^6 hiPSCs were harvested as cell clumps and re-suspended in a 2:1 mixture of hiPSC media:Matrigel (BD Biosciences, San Jose, CA, USA) in a final volume of 150 μl. Prepared

hiPSCs were injected with a 21G needle, intra-muscularly (gastrocnemius) into male NOD SCID mice (4 weeks old) (Charles River Laboratories International Inc, Wilmington, MA). Mice were killed after approximately eight weeks when tumors were detected by palpation. Teratomas were excised, fixed, paraffin sectioned and stained with hematoxylin and eosin (H&E). Teratoma sections were also stained for markers representing the three germ layers: β III TUBULIN (ectoderm) (1:100; ab52901), α ACTININ (mesoderm) (1:100; ab18061; Abcam, Cambridge, MA, USA) and AFP (endoderm) (1:100; A8452; Sigma-Aldrich Corp., St. Louis, MO, USA). All experiments on animals were performed in accordance with NIH guidelines ('Principles of laboratory animal care'; NIH publication no. 85-23, revised 1985) and following approval by Institutional Animal Care and Use Committee of the Joslin Diabetes Center.

Cytogenetic analysis

G-banded karyotyping was performed by the Cytogenetics Core of Dana Farber Harvard Cancer Center (P30 CA006516). Metaphase cells for karyotype analysis were obtained from hiPSCs by incubation with 0.02 ug/ml of colcemid (Irvine Scientific, Santa Ana, CA, USA) for 3-16 h at 37 °C. Cells were trypsinized, collected in a 15 ml tube, and exposed to hypotonic (0.075 M KCl) for 20 min at 37 °C. Cells were fixed in methanol:glacial acetic acid (3:1) three times and then dropped onto slides for chromosome spreads. The slides were baked overnight at 60 °C, treated with trypsin for 30 s and stained with Giemsa for GTG bands.

SUPPLEMENTAL FIGURE LEGENDS

SUPPLEMENTAL FIGURE 1. Derivation of hiPSCs from healthy individuals and MODY patients. MODY gene mutations were confirmed in A) MODY1 (p.Ile271fs), B) MODY2 (V62A), C) MODY3 (P291fsinsC), D) MODY5 (S148L), and E) MODY8 (C563fsX673) hiPSCS. F) Reprogramming efficiencies for the various individual skin fibroblasts were determined by dividing number of hPSC-like colonies by the number of fibroblasts that were initially seeded and transduced (3×10^4 cells). G) Immunostaining analyses for β III TUBULIN (ectoderm), α ACTININ (mesoderm) and AFP (endoderm) in teratoma sections. Scale bar: 100 μm. H) Determination of copy number (lenti)viral integration in hiPSC lines as compared to a healthy control fibroblast with two copies of *SOX2* allele.

SUPPLEMENTAL FIGURE 2. Characterization of hiPSCs. Immunostaining analyses for OCT4, SOX2, NANOG, SSEA-4 and TRA-1-60 in a control hESC line (CHB8) and various hiPSC lines (iAG16102, i120111, iN904-2, iN904-6, iN2-9, iN26-3, iN26-7, iN805-2, iN805-6, iN919-2 and iN65-51). 4',6-diamidino-2-phenylindole (DAPI) is a fluorophore which stains DNA in the nucleus. Scale bar: 100 μm.

SUPPLEMENTAL REFERENCES

1. Raeder, H., Bjorkhaug, L., Johansson, S., Mangseth, K., Sagen, J. V., Hunting, A., Folling, I., Johansen, O., Bjorgaas, M., Paus, P. N., Sovik, O., Molven, A., and Njolstad, P. R. (2006) A hepatocyte nuclear factor-4 alpha gene (HNF4A) P2 promoter haplotype linked with late-onset diabetes: studies of HNF4A variants in the Norwegian MODY registry. *Diabetes* **55**, 1899-1903
2. Sagen, J. V., Bjorkhaug, L., Molnes, J., Raeder, H., Grevle, L., Sovik, O., Molven, A., and Njolstad, P. R. (2008) Diagnostic screening of MODY2/GCK mutations in the Norwegian MODY Registry. *Pediatr Diabetes* **9**, 442-449
3. Bjorkhaug, L., Sagen, J. V., Thorsby, P., Sovik, O., Molven, A., and Njolstad, P. R. (2003) Hepatocyte nuclear factor-1 alpha gene mutations and diabetes in Norway. *J Clin Endocrinol Metab* **88**, 920-931
4. Haldorsen, I. S., Vesterhus, M., Raeder, H., Jensen, D. K., Sovik, O., Molven, A., and Njolstad, P. R. (2008) Lack of pancreatic body and tail in HNF1B mutation carriers. *Diabet Med* **25**, 782-787
5. Raeder, H., Johansson, S., Holm, P. I., Haldorsen, I. S., Mas, E., Sbarra, V., Nermoen, I., Eide, S. A., Grevle, L., Bjorkhaug, L., Sagen, J. V., Aksnes, L., Sovik, O., Lombardo, D., Molven, A., and Njolstad, P. R. (2006) Mutations in the CEL VNTR cause a syndrome of diabetes and pancreatic exocrine dysfunction. *Nat Genet* **38**, 54-62
6. Park, I. H., and Daley, G. Q. (2009) Human iPS cell derivation/reprogramming. *Curr Protoc Stem Cell Biol* **Chapter 4**, Unit 4A 1
7. Sommer, C. A., Sommer, A. G., Longmire, T. A., Christodoulou, C., Thomas, D. D., Gostissa, M., Alt, F. W., Murphy, G. J., Kotton, D. N., and Mostoslavsky, G. (2010) Excision of reprogramming transgenes improves the differentiation potential of iPS cells generated with a single excisable vector. *Stem Cells* **28**, 64-74